JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

A Comparative Study on Antidiabetic Activity of *Gymnema Sylvestre*, Saxagliptin, Insulin and Alloherbal Combination in Alloxan Induced Diabetic Rats

PRATIK V. RANAWARE *, VITTHAL J. CHAWARE, ASHISH T. THORAT, VIVEKKUMAR K. REDASANI. *Department of Pharmacology, YSPM's Yashoda Technical Campus Faculty of pharmacy, Wadhe, Satara, India.*

ABSTRACT

Aim of the study **:** In this study, we evaluated and compared the effect of *Gymnema sylvestre*, Saxagliptin, Insulin and Alloherbal combination (*Gymnema sylvestre* & Saxagliptin) on hyperglycemia, plasma lipid profile, liver enzymes in Alloxan induced diabetes mellitus in rats.

Method**:** The antidiabetic activity (along with other parameters) of *Gymnema sylvestre* (100mg/kg), Saxagliptin (25mg/kg), Insulin (Human Actrapid; 10U/kg), Alloherbal combination (Gymnema sylvestre; 100mg/kg & Saxagliptin; 25mg/kg) was investigated in alloxan induced diabetes in rats. These drugs were administered once a day, for 14 days and blood glucose levels were measured on 0, 7 and 14th day. At the end of treatment various biochemical estimations & histopathological examination of pancreas were also carried out.

Result: The statistical data indicated, 14 Days oral administration all drugs included in study showed significant ($P<0.05$) decreased in blood glucose, total cholesterol, triglycerides, LDL; SGPT and SGOT level , along with significant increase in HDL; But not better than Alloherbal combination.

Conclusion : Present research findings provide experimental evidence that the combination of allopathic hypoglycemic drug; Saxagliptin with hypoglycemic herbal drug; *Gymnema sylvestre* provides effective and rapid glycemic control on diabetes mellitus and it could be considered for further evaluation in clinical studies and drug development.

Key words: *Gymnema sylvestre*, Saxagliptin, Insulin, Alloxan, Alloherbal combination, Diabetes

1. INTRODUCTION

Diabetes mellitus (DM) It is a metabolic disorder characterized by hyperglycaemia, (fasting plasma glucose ≥ 126 mg/dL and/or > 200 mg/dL 2 hours after 75 g oral glucose), glycosuria, hyperlipidaemia, negative nitrogen balance and sometimes ketonaemia. A widespread pathological change is thickening of capillary basement

membrane, increase in vessel wall matrix and cellular proliferation resulting in vascular complications like lumen narrowing, early atherosclerosis, sclerosis of glomerular capillaries, retinopathy, neuropathy and peripheral vascular insufficiency.**[1]**

***Gymnema sylvestre*[2]** is a perennial woody vine native to Asia (including the Arabian Peninsula), Africa and Australia. It has been used in Ayurvedic medicine. Common names include gymnema,**[3]** Australian cowplant, and Periploca of the woods, and the Hindi term *gurmar*, which means "sugar destroyer".**[4][5][6]** It has significant antidiabetic as well as hypolipidemic activity so that it can be used as an adjuvant along with allopathic treatment of medicine to treat diabetes as well as to delay the late complications of diabetes.**[7]**

Saxagliptin a Dipeptidyl Peptidase-4 (DPP-4) inhibitor are the newer class of compounds that was approved in 2006 for the treatment of T2DM. Their primary mechanism of action is through inhibition of degradation of incretins, such as glucagon like peptide-1 (GLP-1) and Gastric Inhibitory Polypeptide (GIP)**[8]**

Human Actrapid is a fast-acting insulin. Onset of action is within ½ hour, reaches a maximum effect within 1.5–3.5 hours and the entire duration of action is approximately 7–8 hours.The blood glucose lowering effect of insulin is due to the facilitated uptake of glucose following binding of insulin to receptors on muscle and fat cells and to the simultaneous inhibition of glucose output from the liver. [9]

This work reviews and comparatively analyzes the herbal, allopathic and biologic treatments to cure the problems in health care. It suggests the adoption of the concept of integrative medication and health care that connects mainstream allopathic medical treatment, herbal therapies and biologics, which will select the best, scientifically validated therapies out of the systems.

2. MATERIALS AND METHODS:

2.1 Drugs and Chemicals:

Alloxan monohydrate obtained from Dolphin pharmacy instruments Pvt. Ltd. Mumbai. *Gymnema sylvestre* obtained from Inlife pharma Pvt. Ltd. Saxagliptin obtained from CTX Lifesciences Pvt. Ltd. Gujrat. Human Actrapid Insulin 40IU/ml obtained from novo nordisk®

2.2 Animals & Housing Condition :

Albino Wistar Rats of (180-200gm) were selected for experimental study. The animals were kept and maintained under laboratory conditions of temperature 22 ± 2°C, relative humidity 50± 15%and 12 hrs. light/dark cycle. They were allowed free access to food (standard pellets) and water *ad libitum.* Experimental protocols and procedures used in this study were approved by the Institutional Animal Ethics Committee of YSPM's, YTC, Faculty of Pharmacy, NH4 Wadhe, Satara, Maharashtra, India.

2.3 Induction of Diabetes:

Albino Wistar Rats were made diabetic by a single intraperitoneal injection of Alloxan monohydrate (150 mg/kg/day). Alloxan monohydrate solution of 150mg/kg/day prepared in 0.9% NaCl solution and was administered within 5 minutes at a dose of 150-mg/kg/day intraperitoneally. All the animals except control group were i.p. administered with Alloxan at a dose of 150mg/kg once a day for 2 days. After 72 hours of Alloxan administration, rats with moderate diabetes having glycosuria and hyperglycemia (i.e. with a blood glucose of 250- 350mg/dl) were taken for the experiment. **[10]**

2.4 Blood Glucose level & Body Weight Determination:

Blood samples were drawn from tail tip of rats. Fasting blood glucose estimation were done on 0^{th}, 7^{th}, & 14^{th} day of the study. Blood glucose estimation was done by ACCU-CHECK Active Glucometer using glucose test strips. For body weight determination, all experimental animals were weighted on 0^{th}, 7^{th}, & 14^{th} day of the study. The body weights were recorded at recording time in the morning mentioned by Al-Attar and Zari **[11]**. Furthermore, for any signs of abnormalities throughout the duration of investigation, the rats were continuously observed.

2.5 Biochemical Estimation:

After Fourteen days, rats were fasted for 8 h. Rats were anesthetized using diethyl ether and samples of blood were obtained from retro-orbital plexus. These Blood samples were withdrawn for estimation of Blood glucose level, Lipid profile (Total cholesterol, Triglycerides, HDL, LDL, VLDL), Liver function test (Alkaline phosphatase, AST;SGOT, ALT;SGPT, Total Protein etc.).

2.6 Histopathological Examination:

After blood collection, all rats were sacrificed with high dose of anaesthesia and dissected; pancreatic tissues were isolated and fixed in 10% formalin. Fixed pancreatic tissues were dehydrated and embedded in paraffin. All tissues were sectioned at 4 µm. The routine process of staining was applied using hematoxylin and eosin stains **[12]**. The pancreatic sections were evaluated by light microscopy using Motic basic biological microscope BA210. Motic imaging software was used to evaluate the histological profile of pancreatic sections in all groups.

2.7 Experimental Design:

2.7.1 Acute Toxicity Study:

Acute toxicity study was carried out for Gymnema sylvestre by adapting fixed dose method of CPCSEA, OECD guidelines no. 423. Healthy Albino Wistar rats of either sex were randomly divided into 4 groups with 3 animals in each group. The animals were kept fasted overnight providing only water, after which the *Gymnema sylvestre* were administered orally with Starting dose is selected from one of four fixed levels 5, 50, 300, and 2000 mg/kg body weight by intra gastrictube. Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily there after, for a total of 14 days. Animals are observed for general neurological & behavioural or autonomic profile. **[13]**

2.7.2 Hypoglycemic Evaluation:

For Hypoglycemic evaluation, Albino Wistar Rats were used and divided into five groups of six animals in each group. Animals were kept fasted overnight (18hrs) before treatment.

Group I - (Control) rats received vehicle that was Distilled water (10ml/kg p.o.).

Group II - (Test1) rats received *Gymnema sylvestre* (100mg/kg p.o.)

Group III - (Test2) rats received Saxagliptin (25mg/kg p.o.)

Group IV - (Test3) rats received Insulin (1U/100gm SC).

Group V - (Test4) rats received *Gymnema sylvestre* (100mg/kg p.o.) and Saxagliptin (25mg/kg p.o.) in combination.

Blood glucose was estimated on 0, 30, 60, 90, 120 min of the treatment using the ACCU-CHECK Active Glucometer.

2.7.3 Oral Glucose Tolerance Test:

For OGTT evaluation, Albino Wistar Rats were used and divided into five groups of sixanimals in each group. Animals were kept fasted overnight (18hrs.) before treatment.

Group I- (Control) rats received Glucose (2gm/kg p.o.)

Group II- (Test1) rats received *Gymnema sylvestre* leaves extract (100mg/kg p.o.)

Group III- (Test2) rats received Saxagliptin (25mg/kg p.o.)

Group IV- (Test3) rats received Insulin (1U/100gm SC).

Group V- (Test4) rats received *Gymnema sylvestre* leaves extract (100mg/kg p.o.) andSaxagliptin (25mg/kg p.o.) in combination.

Glucose (2gm/kg p.o.) was administered to all the rats after Half hour of administration of differentdrug treatments. Blood glucose was estimated at 0, 30, 60, 90 & 120 min after different drug treatment using the ACCU-CHECK Active Glucometer.

2.7.4 Antidiabetic study by different drug treatment :

After 72 hours of Alloxan (150mg/kg/day i.p.) administration, rats with moderate diabetes havingglycosuria and hyperglycemia (i.e. with a blood glucose of 250-350 mg/dl) were taken for the experiment. The Albino Wistar rats were divided into six groups of six rats in each group. All the animals were fasted overnight (18hrs.) before the treatment of test drug.

Group I- (Normal Control) rats received only vehicle that is Distilled water (10ml/kg/day)

Group II- (Toxic Control) rats received Alloxan Monohydrate (150mg/kg/day)

Group III- (Test 1) rats received *Gymnema sylvestre* leaves extract (100mg/kg p.o.)

Group IV- (Test 2) rats received Saxagliptin (25mg/kg/day p.o.)

Group V- (Test 3) rats received Insulin (1U/100gm/day SC).

Group VI- (Test 4) rats received *Gymnema sylvestre* leaves extract (100mg/kg/day p.o.) and Saxagliptin (25mg/kg/day p.o.) in combination.

2.8 Statistical Analysis :

All values of results were presented as mean ± standard error of mean (SEM). The statistical analysis involving one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison posttest was used for statistical comparison between control and various treated groups. Statistical significance was accepted at the $p < 0.05$ values.

3. RESULTS

3.1 Hypoglycemic Effect of different drug treatment in Normal Rats:

The results from the study clearly indicated that the administration of *Gymnema sylvestre*, Saxagliptin, Insulin and *Gymnema sylvestre* with Saxagliptin (combination) reduced the blood glucose level significantly on 90 and 120min as compared with normal control group.

Table 1: Hypoglycemic Effect of different drug treatment in Normal Rats

Group no.	Treatment Groups (n=6)	Fasting Blood Glucose Level (mg/dl)				
		0 min	30min	60min	90min	120min
I	NormalControl	67±1.78	70±1.89	66±1.88	67±3.10	66±2.29
II	Test group 1 (*Gymnema sylvestre*)	73±1.73	71±2.11	69±2.36	65±1.92	60±3.15
III	Test group 2 (Saxagliptin)	81±2.43	71±2.39	63±2.01	57±2.36	55±1.87
IV	Test group 3 (Insulin)	90±2.46	68±1.89	53±2.79	56±2.30	48±3.48
V	Test group 4 (G.S + Saxagliptin)	71±2.03	65±2.76	63±3.07	60±2.88	58±2.15

Values are mean ± SEM, n = 6, when compared with normal by using one way ANOVA followed by Dunnett's multiple comparison test. ($^{*}p < 0.05$).

3.2 Effect of different drug treatment on the Oral Glucose Tolerance Test in Normal Rats:

The results from the study, clearly indicated that the administration of *Gymnema sylvestre*, Saxagliptin, Insulin and *Gymnema sylvestre* with Saxagliptin (combination) reduced the blood glucose level (hyperglycemia due to glucose load (2g/kg p.o.) significantly after 120 min of administration, as compared with control group.

Table 2: Effect of different drug treatment on the Oral Glucose Tolerance Test in NormalRats

Group no.	Treatment Groups (n=6)	Fasting Blood Glucose Level (mg/dl)				
		0 min	30min	60min	90min	120min
I	Control (Glucose)	80±1.54	78±2.55	130±1.67	110±1.89	94±1.56
II	Test group 1 (*Gymnema sylvestre*)	85±1.25	82±1.99	120±3.89	109±2.56	90±1.59
III	Test group 2 (Saxagliptin)	88±1.55	83±2.45	110±1.98	95±1.22	85±3.68
IV	Test group 3 (Insulin)	87±1.98	70±3.56	82±1.36	62±2.66	41±3.02
V	Test group 4 (G.S + Saxagliptin)	90±1.5	83±3.01	111±2.65	95±1.98	82±1.75

Values are mean ± SEM, n = 6, when compared with normal by using one way ANOVA followed by Dunnett's multiple comparison test. ($^{*}p < 0.05$).

3.3 Effect of different drug treatment on Body Weight of Diabetic Rats:

At the end of study after 14 days, body weight was significantly decreased in toxic control groupas compared with normal control group & significantly increased in *Gymnema sylvestre*, Saxagliptin, Insulin and *Gymnema sylvestre* with Saxagliptin (combination) treated group as compared with toxic control group. But Insulin treated group shows marked rise in body weight than the Normal control group.

Table 3: Effect of different drug treatment on Body Weight of Diabetic Rats

Group no.	Treatment Groups (n=6)	Body Weight of Animals (gm)		
		0^{th} day	7^{th} day	14^{th} day
I	Normal control	210±1.33	215±1.89	224±2.01
II	Toxic control	216±1.65	196±2.96	180±3.69
III	Test group 1	228±2.63	232±3.06	238±1.32
IV	Test group 2	237±3.01	242±1.65	249±2.36
V	Test group 3	241±2.65	252±3.11	266±1.158
VI	Test group 4	248±1.65	253±2.36	259±3.05

Values are mean ± SEM, n = 6, when compared with normal by using one way ANOVA followed by Dunnett's multiple comparison test. ($^{*}p < 0.05$).

3.4 Effect of different drug treatment on Fasting Blood Glucose Level in Diabetic Rats

A marked rise in fasting blood glucose level was observed in toxic control group as compared with normal control group. *Gymnema sylvestre* (100mg/kg) And Saxagliptin (25mg/kg) treated group which produced a significant reduction in blood glucose level as compared with toxic control group; But not better than their combination. Where the Insulin treated group shows Goodcontrol of Hyperglycemia until 7th day then it shows mild Hypoglycemia on 14th day.

Table 4: Effect of different drug treatment on Fasting Blood Glucose Level in Diabetic Rats

Group no.	Treatment Groups (n=6)	Fasting Blood Glucose Level (mg/dl)		
		0th day	7th day	14th day
I	Normal Control	80±1.89	80±1.045	82±2.07
II	Toxic control	321±2.89	326±1.22	330±1.65
III	Test group 1 (*Gymnema sylvestre*)	285±1.59	221±1.03	157±2.67
IV	Test group 2 (Saxagliptin)	299±2.86	191±1.09	144±1.75
V	Test group 3(Insulin)	325±1.65	195±2.69	65±1.44
VI	Test group 4 (G.S + Saxagliptin)	316±1.07	165±1.76	115±2.66

Values are mean ± SEM, n = 6, when compared with normal by using one way ANOVA followed by Dunnett's multiple comparison test. ($^{*}p < 0.05$).

3.5 Effect of different drug treatment on Biochemical Parameters in Diabetic Rats:

Serum Lipid Profile:

After 14 days of treatment period it was observed that increased level of Total Cholesterol, TG, LDL, VLDL, & decreased HDL level in toxic control group as compared with normal control group. Animals treated with *Gymnema sylvestre* (100mg/kg) And Saxagliptin (25mg/kg) showedsignificant reductions in Total Cholesterol, LDL, VLDL, TG & significant increased level in HDLas compared with toxic control group; But not better than their combination (i.e. G.S + Saxagliptin). Where Test group 3 (i.e. Insulin) showed reduction in values than normal control values.

Table 5 : Effect of different drug treatment on Biochemical Parameters in Diabetic Rats

Group no.	Treatment Groups (n=6)	Total Cholesterol (mg/dl)	Tri-Glycerides (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)
I	NormalControl	78.4±1.54	122±2.03	11.9±1.98	39±1.09	24±1.45
II	Toxic control	102±2.13	480±1.98	38±3.54	35±4.03	96±1.84
III	Test group 1 (*Gymnema sylvestre*)	87±3.05	153±2.03	18.3±1.04	37±4.65	30.7±2.51
IV	Test group 2 (Saxagliptin)	100±2.65	140±3.98	24.8±1.75	34±4.03	28±1.33
V	Test group 3 (Insulin)	66±2.68	201±1.66	21±3.78	30±4.65	40.2±1.21
VI	Test group 4 (G.S + Saxagliptin)	83±1.03	120±2.78	19±1.98	39±3.98	24±4.01

Values are mean ± SEM, n = 6, when compared with normal by using one way ANOVA followedby Dunnett's multiple comparison test. ($^{*}p < 0.05$).

3.6 Effect of different drug treatment on Liver Function Test in Diabetic Rats :

After 14 days of treatment period it was observed that increased level of Bilirubin, SGPT, SGOT,TP & ALKP in toxic control group as compared with normal control group. Animals treated with*Gymnema sylvestre* (100mg/kg) And Saxagliptin (25mg/kg) showed significant reductions in AST; SGOT, ALT; SGPT & ALP as compared with Toxic control group; But not not better thantheir combination (i.e. G.S + Saxagliptin). Where Test group 3 (i.e. Insulin) shows reduction in values than normal Control values.

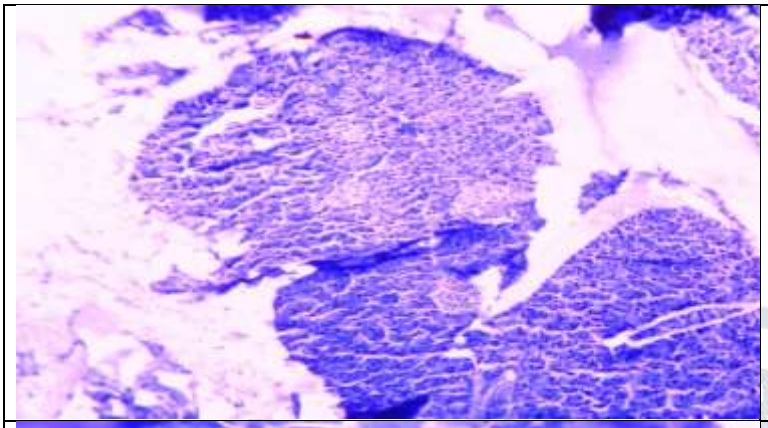
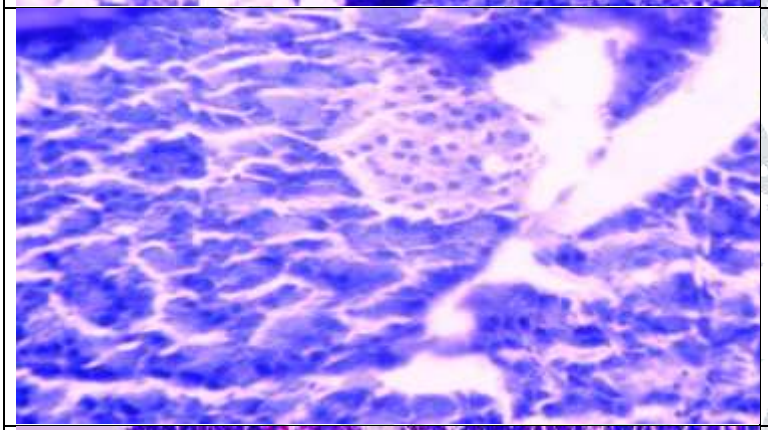
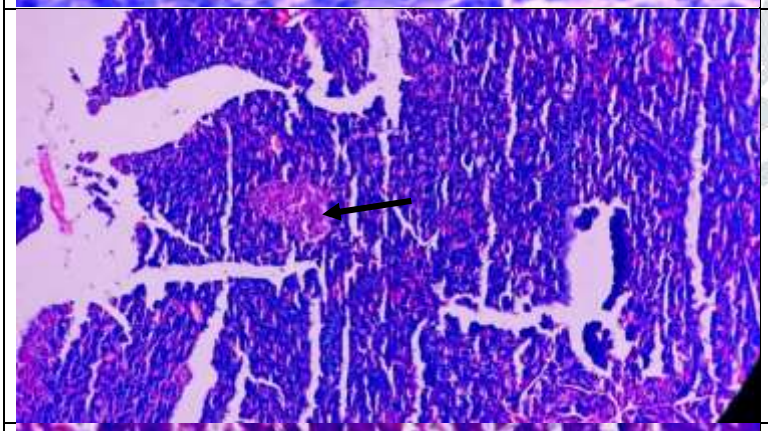
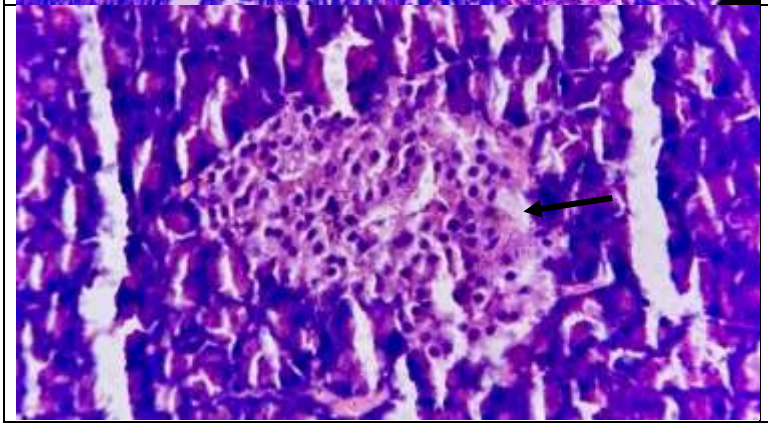
Table 6 : Effect of different drug treatment on Liver Function Test

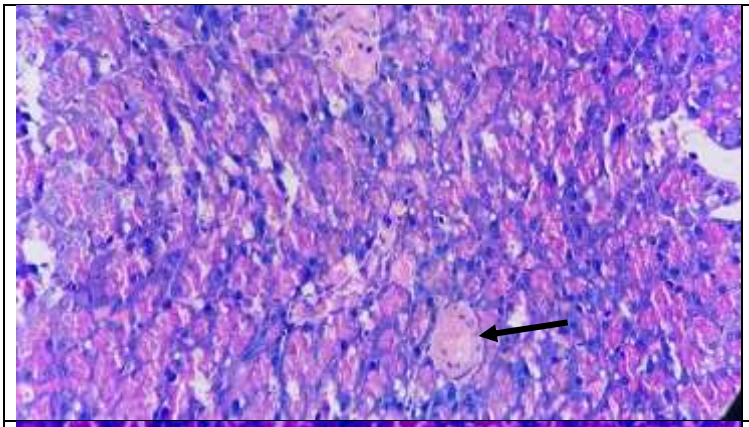
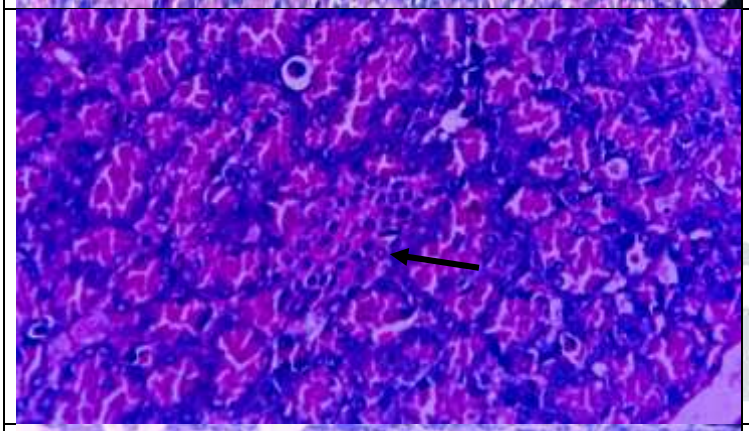
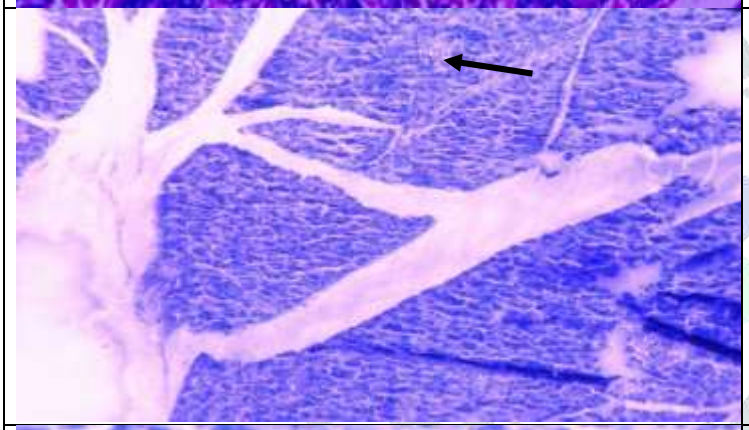
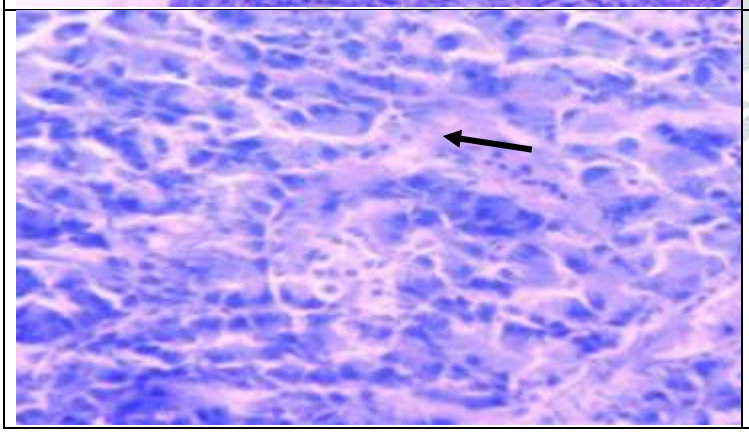
Group no.	Treatment Groups (n=6)	Bilirubin mg/dl	SGPT U/L	SGOT U/L	TP mg/dl	ALKP U/L
I	NormalControl	0.74±1.33	43±1.6	36±3.06	5.8±2.09	126±1.48
II	Toxic control	1.12±2.35	59±1.59	42±3.65	7.5±4.89	190±2.89
III	Test group 1 (*Gymnema sylvestre*)	0.83±1.78	46±2.13	36±3.60	6.5±4.21	110±2.44
IV	Test group 2 (Saxagliptin)	0.93±2.54	38±3.45	31±1.09	6.9±4.01	102±1.98
V	Test group 3 (Insulin)	0.6±1.08	34±2.40	29±1.88	4.9±4.02	97±3.48

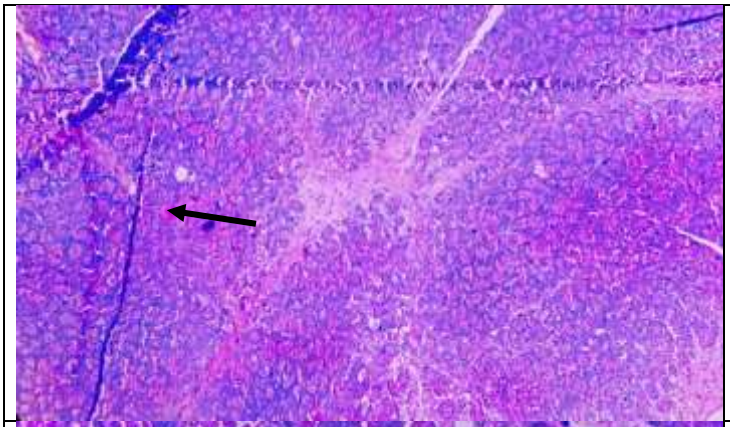
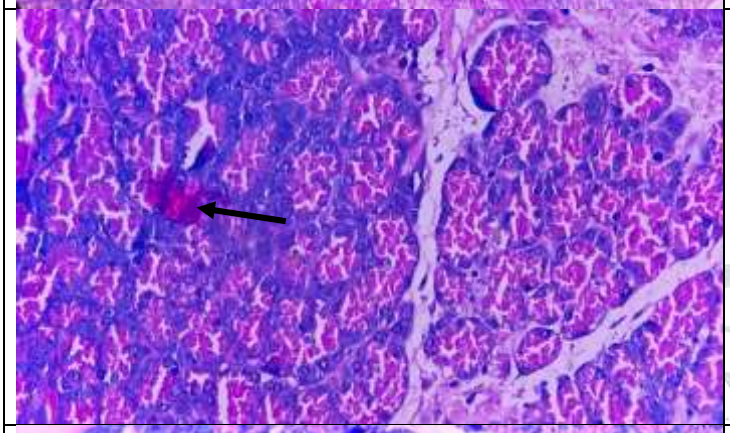
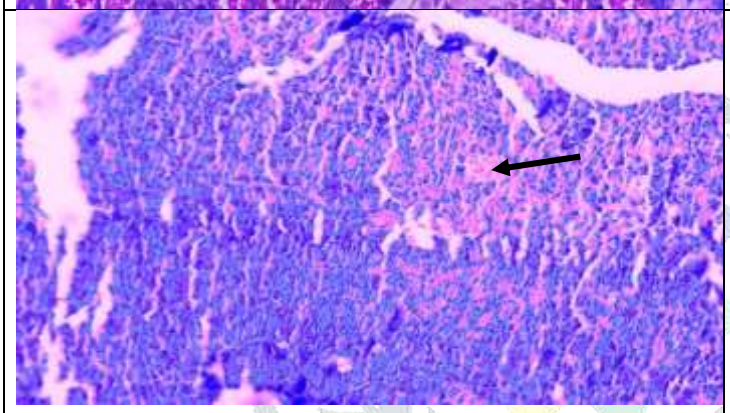
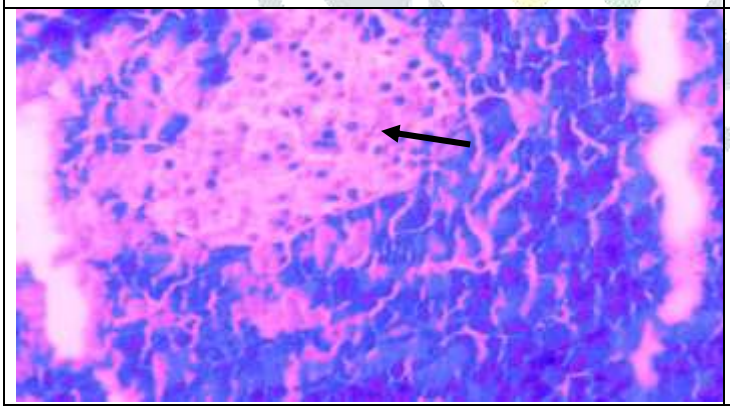
VI	Test group 4(G.S + Saxagliptin)	0.79±1.03	39±2.03	30±2.78	6.9±3.33	118±4.11

Values are mean ± SEM, n = 6, when compared with normal by using one way ANOVA followed by Dunnett's multiple comparison test. ($^{*}p < 0.05$).

Pancreas Histopathology

	**Normal control Pancreas** –H & E stain 10X
	**Normal control Pancreas** –H & E stain 40X
	**Alloxan inducer group** - Photograph showing necrosis of islets of Langerhans (black arrow) H & E stain 10X
	**Alloxan inducer group** - Photograph showing necrosis of islets of Langerhans with cellular infiltration H & E stain 40X

	**Test group 1 –** Photograph showing Acinar and degeneration of islet of Langerhans (Black Arrow), H & E stain 10X
	**Test group 1 –** Photograph showing Acinar and degeneration of islet of Langerhans (Black Arrow), H & E stain 40X
	**Test group 2** – Photograph showing necrosis of islets of Langerhans (black arrow),H & E stain 10X
	**Test group 2** – Photograph showing necrosis of islets of Langerhans with cellular infiltration (black arrow) H & E stain 40X

	**Test group 3** – Photograph showing Acinar Hemorrahage (black arrow) H & E stain 10X
	**Test group 3** – Photograph showing Acinar hemorrhage and degeneration ofislet of Langerhans with Hyalination (Black Arrow), H & E stain 40X
	**Test group 4** – Photograph showing islets of Langerhans (black arrow) H & E stain 10X
	**Test group 4** – Photograph showing islets of Langerhans with Acinar degeneratiom H & E stain 40X

4. DISCUSSION :

4.1 Acute oral toxicity, Hypoglycemic study, OGTT Study & Body Weight Determination:

Globally, the rapid increase the incidence of DM poses a demand for the quest of novel therapeuticdrugs necessitates addition of alternative medicine. As a result number of studies has been conductedto assess the utility of herbal and allopathic medicine in DM. The present study was undertaken to evaluate the Antidiabetic activity of *Gymnema sylvestre,* Saxagliptin, Insulin and Alloherbal combination against Alloxan Induced Diabetic Albino Wistar Rats.

The Acute oral toxicity was performed according to OECD guideline 423. In this study we observed that the *Gymnema sylvestre* was safe to use in animals. There was no change in neurological, behavioural or autonomic, no lethality or toxic reactions were found with the selecteddoses (5, 50, 300 and 2000mg/kg/day p.o.) until the end of study period. Therefore 100 mg/kg was selected for all in vivo experiments as minimal dose.

The results of Hypoglycemic study have shown that the administration of *Gymnema sylvestre*, Saxagliptin, Insulin and *Gymnema sylvestre* with Saxagliptin (combination) reduced the blood glucose level significantly on 120min as compared with normal control group.(Table 3).

OGTT for nondiabetic rats were performed according to the standard method (Du Vigneaud and Karr, 1925).**[14]** The Oral glucose tolerance test in nondiabeticc rats, blood glucose level was significantly greater in the glucose loaded control group. The results from the study, clearly indicated that the administration of *Gymnema sylvestre*, Saxagliptin, Insulin and *Gymnema sylvestre* with Saxagliptin (combination) reduced the blood glucose level (hyperglycemia due to glucose load (2gm/kg p.o.) after120 min of administration, as compared with control group. (Table 4).

Induction of diabetes by Alloxan leads to loss of body weight due to increased muscle wasting and loss of tissue proteins as well as due to destruction of pancreatic cells; insufficient insulin prevents the body from getting glucose from the blood into the body's cells to use as energy & when this occurs, the body starts burning fat and muscle for energy, causing a reduction in overall body weight.)**[15]** whereas body weight of animals significantly increased in *Gymnema sylvestre*, Saxagliptin and their combination treated group as compared with toxic control group. But treatmentof Insulin shows marked rise in body weight.).**[16]** (Table 5).

4.2 Alloxan-Induced Rodent Model of Diabetes & Antidiabetic effect of different drug treatment:

Alloxan has two distinct pathological effects: Alloxan is a toxic glucose analogue it selectively inhibits glucose-induced insulin secretion through specific inhibition of glucokinase, the glucose sensor of the beta cell, and it causes a state of insulin-dependent diabetes through its ability to induceROS formation leading to demolition of pancreas β-cells & selective necrosis leading to hypoinsulinemia and hyperglycemia.

The results of the antidiabetic study reduced blood glucose level Significantly on 7^{th} & 14^{th} days when animals treated with *Gymnema sylvestre*, Saxagliptin, Insulin and *Gymnema sylvestre* with Saxagliptin (combination) as compared to toxic control groups (Table 6)

There are some possible mechanisms by which the *Gymnema sylvestre* leaves and especially Gymnemic acids from G. sylvestre exert its hypoglycemic effects are: 1) it increases secretion of insulin, 2) it promotes regeneration of islet cells, 3) it increases utilization of glucose: it is shown toincrease the activities of enzymes responsible for utilization of glucose by insulin dependant pathways, an increase in phosphorylase activity, decrease in gluconeogenic enzymes and sorbitol dehydrogenase, and 4) it causes inhibition of glucose absorption from intestine).**[17]**

Saxagliptin is part of a class of diabetes medications called dipeptidyl peptidase-4 (DPP-4) inhibitors. DPP-4 is an enzyme that breaks down incretin hormones. As a DPP-4 inhibitor, saxagliptin slows down the breakdown of incretin hormones, increasing the level of these hormonesin the body. It is this increase in incretin hormones that

is responsible for the beneficial actions of saxagliptin, including increasing insulin production in response to meals and decreasing the rateof gluconeogenesis in the liver).**[18]** Dipeptidyl peptidase-4's role in blood glucose regulation is thought to be through degradation of GIP and the degradation of GLP-1).**[19][20]**

The blood glucose lowering effect of insulin is due to the facilitated uptake of glucose following binding of insulin to receptors on muscle and fat cells and to the simultaneous inhibition of glucose output from the liver).**[21]**

4.3 Biochemical Parameters Analysis:

In Alloxan induced diabetes mellitus showed improvement in biochemical parameters after the treatment of Drugs.

In the result of **lipid profile**, marked decrease in total cholesterol, LDL, VLDL and triglycerides was observed, while increase in HDL cholesterol which reduces the risk of atherosclerosis has been observed in *Gymnema sylvestre*, Saxagliptin, Insulin and *Gymnema sylvestre* with Saxagliptin (combination) treated diabetic rats, which suggest that HDL is inversely related to the total body cholesterol as compared with toxic control group (Table 7).

These results could thus reflect the ability of *Gymnema sylvestre*, Saxagliptin improve the tissue sensitivity to insulin. Thus reducing the hormone sensitive lipase activity and increasing the lipoprotein lipase activity, resulting in a decrease of lipolysis these leading to hypolipidemic activity.

In the present study, rats treated with G. sylvestre post Alloxan-diabetic induction showed a significant decrease in triglyceride, cholesterol and LDL and showed a significant increase in HDLas compared to that of untreated diabetic rats. Decreasing levels of triglyceride, cholesterol and LDLand increasing level of HDL might be due to an increase in insulin which caused an increased activity of lipoprotein lipase (Facilitated chylomicron transport through cell membranes) and a decreased activity of hormone-sensitive lipase (converted neutral fats into free fatty acids). This result was in agreement with Daisy et al. (2009) **[22]** and Aralelimath and Bhisea (2012) **[23]** who reported that increasing insulin secretion after administration of G. sylvestre extract led to a decreaseof cholesterogenesis and fatty acid synthesis.

Our study results elucidated that, saxagliptin improve lipid status in rats, via significant reduction of Total Cholesterol, LDL and Triglycerides. In line with these results are also the results of other research groups. Possible explanation for beneficial lipid effects of DPP4 inhibitors may be connected to its stimulating effect on the activated proteine-kinase pathway, which leads to increasein glucose and lipid catabolism. **[24]** On the other hand, no improvement in HDL parameters was achieved in our study, which is in correlation with the findings of Saad et al. **[25]**

In our study the result of Insulin treatment is accordance with Ibrahim Aslan et.al. (2013) **[26]** whichshows Total cholesterol (TC), triglyceride (TG) and very low-density lipoprotein cholesterol (VLDL-C) levels were significantly decreased while HDL-C levels were significantly increased after insulin treatment.

In **liver function test**, animals treated with *Gymnema sylvestre*, Saxagliptin, Insulin and *Gymnema sylvestre* with Saxagliptin (combination) treated group showed significant reductions in Bilirubin, ALT; SGPT, AST; SGOT, Total Protein & ALKP as compared with toxic control group (Table 8). This demonstrated the hepatoprotective activity could be related to reduced blood glucose level due to different treatment groups.

Evidence from studies about heme oxygenase (HO) system**[27][28]** might also support the increasedrisk of bilirubin with T2D. Increased activity of HO could elevate the heme catabolic products suchas carbo monoxide, iron, and bilirubin. **[27]** HO-1 has been reported as a strong positive predictor ofmetabolic inflammation among obese insulin-resistance individuals and animals.**[28][29]** The higher bilirubin levels might be a biomarker of oxidative stress and inflammation in diabetes

Glucose level might be decreased in treated diabetic rats as a result of decreasing gluconeogenesis that was indicated by low levels of ALT;SGPT and AST;SGOT in treated diabetic rats compared tountreated diabetic rats(Toxic Control Group). This result was in agreement with Shanmugasundaramet al. (1983)**[30]** who reported that administration of dried leaf powder of G. sylvestre decreased glucose levels as it controlled gluconeogenic enzymes (ALT and AST) and increased glycogen levels in liver, kidney and muscle.

Total proteins were found to be significantly increased in diabetics as compared to Normal controls.Competition between serum albumin and hemoglobin could be a factor for the negative correlationbetween them, besides preventing other proteins from glycation and altering the diabetic complications. Similar findings have been reported by other studies.**[31] [32] [33] [34]** Increase in total proteins may be due to the elevation of acute phase proteins, globulins, fibrinogen and compounded by decrease in the fractional synthetic rate of albumin due to insulin resistance/deficiency (F A Nazki 2017)

4.4 Histopathological Examination:

In histopathological study (Table 19), the fine section of Normal Control diabetic rat's pancreas onmicroscopic examination using H & E stain, 10X & 40X showed the presence of islets of Langerhans, blood vessels, connective tissues, inter and arrangement of islets of Langerhans was normal with tightly arranged cells and even distribution throughout the lob-necrosis.

Also, Pancrease Exocrine portion predominantly and composed of lobules, each of which is surrounded by connective tissue septa through which run blood vessels, nerves, lymphatics, and interlobular ducts. Adequate islets of beta and alfa cells was seen. No evidence of stromal Infiltrationwas seen.

In toxic control group i.e Alloxan inducer group Histopathological report shows that necrosis of islets of Langerhans was shown with cellular infiltration. It also Shows Exocrine portion predominantly and composed of lobules formed by acinar structure, each of which is surrounded byconnective tissue septa through which run blood vessels, nerves, lymphatics, and interlobular ducts.

There is No evidence of islets of beta and alfa cells are seen, which complete destruction of alpha and beta cells.

In Saxagliptin treated diabetic rats, Photograph showing necrosis of islets of Langerhans with cellular infiltration (The migration of cells from their sources of origin), Where In Insulin treated rats showing Acinar hemorrhage

and degeneration of islet of Langerhans with Hyalinization (process of conversion of stromal connective tissue into a homogeneous, acellular translucent material that could provide insights into the prognosis of pathological lesions.) was seen. So it can be concluded that Insulin treatment does not provide protection to the pancreatic cells.

In *Gymnema sylvestre* and *Gymnema sylvestre* with Saxagliptin treated groups it was observed thatalthough the gap between the islets was more than lesser number of islets as compared to Normal control group, it was significantly much better than the toxic control group. By showing Acinar degeneration it is concluded that, the dose of *Gymnema sylvestre* had slight protection to the cells. Because Necrosis formed after Acinar degeneration which was shown in Toxic control group. Thus the histopathological examination revealed good protective property of this herbal drug alone and with combination.

5. CONCLUSION :

In conclusion, it can be stated that the use of Combination of *Gymnema sylvestre* and Saxagliptin produces more beneficial effect than using alone. Gymnemic acids from G. sylvestre exert its hypoglycemic effectsby increasing secretion of insulin, increasing utilization of glucose or inhibiting glucose uptake from intestine; Whereas Saxagliptin exerts its hypoglycemic effect by this increase in incretin hormonesand increasing insulin production in response to meals and decreasing the rate of gluconeogenesis inthe liver.

Use of *Gymnema sylvestre* and Saxagliptin in combination shown beneficial effects in reducing theelevated blood glucose level as well as gained body weight, hypoglycemic potential, significant oral glucose tolerance & normalization in altered biochemical parameters of Alloxan induced diabetic rats.

From the present results it can be concluded that using herbs with allopathic drugs may produce the synergistic effect. Hence, For further use of these combination clinical studies are needed.

6. REFERENCES

1. Tripathi, K. D. (2018). *Essentials of medical pharmacology* (8th ed.). Jaypee Brothers Medical. "Integrated Taxonomic Information System". *www.itis.gov. Retrieved 2018-02-01*

2. Duke JA, ed. (2002). Handbook of medicinal herbs (2nd ed.). CRC Press. p. 855. ISBN 978-0-8493-1284-7.

3. Quattrocchi U (1999-11-23). CRC World Dictionary of Plant Names: Common Names, Scientific Names, Eponyms, Synonyms, and Etymology. *Taylor & Francis US.* ISBN 9780849326769

4. Tiwari P, Mishra BN, Sangwan NS (2014). "Phytochemical and pharmacological properties of *Gymnema sylvestre*: an important medicinal plant". *BioMed Research International*. 2014: 830285. doi:10.1155/2014/830285.

5. Ulbricht C, Abrams TR, Basch E, Davies-Heerema T, Foppa I, Hammerness P, Rusie E, Tanguay-Colucci S, Taylor S, Ulbricht C, Varghese M, Weissner W, Woods J (2011). "An evidence-based systematic review of gymnema (Gymnema sylvestre R. Br.) by the Natural Standard Research Collaboration". *Journal of Dietary Supplements*. 8 (3): 311–30. doi:10.3109/19390211.2011.597977

6. Grijesh Kumar Mall, Pankaj Kishor Mishra* and Veeru Prakash Antidiabetic and Hypolipidemic Activity of *Gymnema sylvestre* in Alloxan Induced Diabetic Rats

7. Vickers SP, Cheetham SC, Birmingham GD, Rowley HL, Headland KR, et al. (2012) Effects of the DPP-4 Inhibitor, Linagliptin, in Diet-Induced Obese Rats:A Comparison in Naive and Exenatide-Treated Animals. ClinLab 58: 787-799.

8. Mandlem VKK1* and Annapurna A2 Cardioprotective Role of Saxagliptin through Antioxidant Mechanism in Experimental Myocardial Infarction in STZ Induced Diabetic Rats. Journal of clinical and experimental pharmacology 2017, 1-2

9. https://www.ema.europa.eu/en/documents/product-information/actrapid-epar-product-information_en.pdf

10. Kamble, U., Chaware, V. J., & Redasani, V. K. (2021). Evaluation of Antidiabetic Potential of Eucalyptus Globulus Plant Extract in Dexamethasone-Induced Diabetic Rats. *Asian Journal of Pharmaceutical Research and Development*, *9*(4), 63-74.

11. A. M. Al-Attar and T. A. Zari, "Influences of crude extract of tea leaves, Camellia sinensis, on streptozotocin diabetic male albino mice," Saudi Journal of Biological Sciences, vol. 17, no. 4, pp. 295– 301, 2010.

12. A. M. Al-Attar and F. A. Alsalmi, "Efect of Olea europaea leaves extract on streptozotocin induced diabetes in male albino rats," Saudi Journal of Biological Sciences, vol. 26, no. 1, pp. 118–128, 2019

13. https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd_gl423.pdf

14. Md. Ariful islam, most. Afia akhtar, md. Rafiqul islam khan, Md. Sarowar hossain, a. H. M. Khurshid alam, mir imam ibne wahed, Md. Shah amran, bytul mokaddesur rahman and maruf ahmed* Oral Glucose Tolerance Test (ogtt) In Normal Control And Glucose Induced Hyperglycemic Rats With Coccinia Cordifolia L. And Catharanthus Roseus l. Article *in* pakistanjournal of pharmaceutical sciences · january 2009; 3

15. https://www.diabetes.co.uk/symptoms/unexplained-weight-loss.html#:~:text=In%20people%20with%20diabetes%2C%20insufficient,reduction%20in%20overall%20body%20weight.

16. S Skovsø1, J Damgaard2, JJ Fels2, GS Olsen2, XA Wolf2, B Rolin2 and JJ Holst1 Effects of insulin therapy on weight gain and fat distribution in the HF/HS-STZ rat model of type 2 diabetes Article *in* International Journal of Obesity · July 2015, 3

17. Parijat Kanetkar, Rekha Singhal* and Madhusudan Kamat Gymnema sylvestre: A Memoir Recent Advances in Indian Herbal Drug Research 2006; 2

18. https://en.wikipedia.org/wiki/Saxagliptin#cite_note-18

19. Rolf MENTLEIN', Baptist GALLWITZ' and Wolfgang E. SCHMIDT* Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-l(7-36)amide, peptide histidinemethionine and is responsible for their degradation in human serum Eur. J. Biochem. *214*, 829-835 (1993)

20. Bo Ahre´ N, Mona Landin-Olsson, Per-Anders Jansson, Maria Svensson, David Holmes, And Anja Schweizer Inhibition of Dipeptidyl Peptidase-4 Reduces Glycemia, Sustains Insulin Levels, and Reduces Glucagon Levels in Type 2 Diabetes The Journal of Clinical Endocrinology& Metabolism 89(5):2078–2084

21. https://www.ema.europa.eu/en/documents/product-information/actrapid-epar-product-information_en.pdf

22. Pitchai Daisya,∗, James Eliza a, Khanzan Abdul Majeed Mohamed Farookb A novel dihydroxy gymnemic triacetate isolated from Gymnema sylvestre possessing normoglycemic and hypolipidemic activity on STZ-induced diabetic Rats Journal of Ethnopharmacology 126 (2009)339–344

23. Vijayanand R. Aralelimath*, Satish B. Bhisea Anti-Diabetic Effects Of *Gymnema Sylvester* Extract On Streptozotocin Induced Diabetic Rats And Possible B-Cell Protective And Regenerative Evaluations. Digest Journal Of Nanomaterials And Biostructures Vol. 7, No 1, January - March 2012, P. 135 – 142

24. Danny Issa | Vaishali Patel | Arun J. Sanyal Future therapy for non-alcoholic fatty liver diseaseDivision of Gastroenterology, Hepatology and Nutrition, Department of Internal
Medicine, Virginia Commonwealth University School of Medicine, Richmond, VA, USA

25. Mohamed I. Saad,Maher A. Kamel, and Mervat Y. Hanafi Modulation of Adipocytokines Production and Serum NEFA Level by Metformin, Glimepiride, and Sitagliptin in HFD/STZ Diabetic Rats Hindawi Publishing Corporation Biochemistry Research International Volume 2015, Article ID 138134, 7 pages http://dx.doi.org/10.1155/2015/138134

26. Ibrahim Aslan1, Ertan Kucuksayan2 and Mutay Aslan2* Effect of insulin analog initiation therapy on LDL/HDL subfraction profile and HDL associated enzymes in type 2 diabeticpatients Aslan et al. Lipids in Health and Disease 2013, 12:54

27. Nader G. Abraham And Attallah Kappas Pharmacological and Clinical Aspects of Heme Oxygenase doi:10.1124/pr.107.07104.

28. Alexander Jais,1,12 Elisa Einwallner,1,12 Omar Sharif,1,2 Klaus Gossens,3 Tess Tsai-Hsiu Lu,3 Selma M. Soyal,4 Heme Oxygenase-1 Drives Metaflammation and Insulin Resistance in Mouse and Man http://dx.doi.org/10.1016/j.cell.2014.04.043

29. Huang J-Y, Chiang M-T, Chau L-Y (2013) Adipose Overexpression of Heme Oxygenase-1 Does Not Protect against High Fat Diet-Induced Insulin Resistance in Mice. PLoS ONE 8(2): e55369. doi:10.1371/journal.pone.0055369

30. K.R. Shanmugasundaram', C. Panneerselvama, P. Samudramb And E.R.B. Shanmugasundaramb Enzyme Changes And Glucose Utilisation In Diabetic Rabbits: The Effect Of *Gymnema Sylvestre*, R.Br. *Journal Of Ethnopharmacology, 7 (1983) 205-234* ElsevierScientific Publishers Ireland Ltd

31. BN. Malawadi, Usha Adiga. plasma proteins in Type 2 DM.IOSR Journal of biotechnology and biochemistry (IOSR-JBB)

32. Shalbha Tiwari,1 Manish Bothale,2 Imtiaz Hasan,3 Mahesh J. Kulkarni, Association between serum albumin and glycated hemoglobin in Asian Indian subjects. Indian J Endocrinol Metab. 2015 Jan-Feb; 19(1): 52–55.

33. Rodriguez-Segade S, Rodriguez J, Mayan D, Camina F.Plasma albumin concentration is a predictor of HbA1c among type 2 diabetic patients, independently of fasting plasma glucose and fructosamine. Diabetes Care. 2005; 28:437–9. [PubMed]

34. Schmidt MI, Duncan BB, Sharrett AR, Lindberg G, Savage PJ, Offenbacher S, Azambuja MI, Tracy RP, Heiss G: Markers of inflammation and prediction of diabetes mellitus in adults (Atherosclerosis Risk in Communities study): a cohort study. Lancet 1999; 353:1649 – 1652.